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Cerebrospinal Fluid F_2-Isoprostane Levels Are Increased in Alzheimer's Disease

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Postmortem studies have associated Alzheimer's disease (AD) with regionally increased oxidative damage to brain. Lacking, however, is a specific marker of oxidative damage to brain that may be measured during life. We tested the hypothesis that cerebrospinal fluid (CSF) concentrations of F_2-isoprostanes (F_2-IsoPs), stable products of arachidonate peroxidation, are increased in CSF of AD patients. CSF from lateral ventricles (VF) was analyzed from 11 AD patients and 11 control subjects who participated in a rapid autopsy program. VF F_2-IsoP concentrations were significantly elevated in AD patients compared with control subjects (72 ± 7 vs 46 ± 4 pg/ml) and were significantly linearly correlated with brain weight (-0.3 pg/ml/g, $r^2 \simeq 0.32$). These results suggest that quantification of CSF F_2-IsoP concentrations may provide a useful biomarker of central nervous system oxidative damage in AD.

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Regional increases in oxidative damage are a feature of brain tissue obtained post mortem from patients with Alzheimer's disease (AD).[1] However, an objective index of oxidative damage associated with AD that may be assessed during life is lacking. Such a biomarker could have an important impact on the ability to test hypotheses concerning oxidative damage in AD patients by permitting repeated evaluation to follow progression of disease and to quantify response to experimental therapeutic interventions.

Lipid peroxidation is a prominent manifestation of oxidative challenge in brain.[1] Recently, we have shown

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that markers of lipid peroxidation are increased in cerebrospinal fluid (CSF) of AD patients compared with control subjects.[2,3] Although these studies suggest that quantification of lipid peroxidation products in CSF may provide an intra vitam index of oxidative damage to brain, the assays used have shortcomings, including the need for large volumes of CSF and measuring highly reactive molecules, such as 4-hydroxynonenal, that limit their interpretation or widespread application.

Previously, we described a series of prostaglandin F_2-like compounds, termed F_2-isoprostanes (F_2-IsoPs), that are produced by free radical–catalyzed peroxidation of arachidonic acid independent of the cyclooxygenase enzyme.[4] Significant advantages to quantifying F_2-IsoP as an index of oxidative stress are their specificity for lipid peroxidation, their chemical stability, and the relatively small tissue volumes required for their detection. A large body of evidence now exists to show that F_2-IsoP concentration is a reproducible, quantitative index of lipid peroxidation in vivo.[5] In this study, we have tested the hypothesis that F_2-IsoP concentrations are increased in CSF of AD patients.

Subjects and Methods

CSF from 24 different subjects was collected after appropriate informed consent was obtained. Twenty-two subjects had autopsies performed in 1996 or 1997. All AD patients had been diagnosed with probable AD during life. Control subjects were age-matched individuals without clinical evidence of dementia or other neurological disease; each of these individuals had annual neuropsychological testing with all test scores in the normal range. Ventricular CSF (VF) was collected from each subject as part of a rapid autopsy protocol. Mean postmortem intervals were 2.9 ± 0.3 hours in control subjects and 2.7 ± 0.2 hours in AD patients; all samples were collected within 4.5 hours of death. Apolipoprotein E (ApoE) genotype was determined post mortem in all cases.[6]

Immediately after aspiration, VF was sedimented at 1,000 *g* for 10 minutes and 1 to 2 ml were frozen at −80°C. There was no visual contamination of aspirates with blood, nor was apolipoprotein B detected in immunoblots of VF.[3] Brains were evaluated by using standard criteria.[7,8] Patients with brainstem or cortical Lewy body formation, or significant cerebrovascular disease were excluded. Control subjects demonstrated only age-associated alterations. Braak staging was performed on all cases.[9]

CSF aspirated intra vitam from the lumbar cistern (LF) was analyzed in 2 additional patients. Both of these patients were being evaluated for neurological disease and LF was obtained for diagnostic purposes. Both samples were free of contamination by blood and had standard clinical chemistry values within normal ranges. Ultimate diagnoses for these 2 patients were optic neuritis and malignant lymphoma. LF was handled and stored as described for VF.

Free F_2-IsoP in 1 to 2 ml of CSF was quantified by using stable isotope dilution methods, using gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICIMS) as described.[4,5] In 7 patients, we also quantified F_2-IsoP-like compounds that are derived from docosahexaenoic acid, the F_4-neuroprostanes (F_4-NPs); these were quantified by a modification of the above GC/NICIMS method as described.[10]

Hypothesis testing for continuous data was performed with unpaired *t* tests. Discontinuous data were compared with the χ^2 test. Single-dimension linear regression analysis and Spearman's ranked correlation were performed, using Prism 2.0 software.

Results

All 22 VF samples analyzed in this study were from subjects who participated in a rapid autopsy program. Clinical, pathological, and F_2-IsoP data for these 22 cases are presented in the Table. Age and sex ratios were characteristic for patients with late-onset AD and were matched to control subjects. Duration of disease was typical for the group of AD patients. Brain weight was significantly lower whereas Braak stage was significantly higher in AD patients compared with control subjects. ApoE4 frequency in control subjects was similar to the value reported for the general population[11] and was significantly overrepresented in AD patients.[12]

Average VF F_2-IsoP levels in AD patients were significantly increased compared with control subjects (see Table). The ranges of VF F_2-IsoP values were 12 to 68 pg/ml in control subjects and 46 to 137 pg/ml in AD patients. Single-dimension linear regression analysis demonstrated a significant correlation between F_2-IsoP levels and brain weight (-0.3 pg/ml/g, $r^2 = 0.32$, $p < 0.01$; Fig), but not with subjects' age ($r^2 = 0.06$), body weight (0.04), or postmortem interval ($r^2 = 0.01$). F_2-IsoP levels tended to increase with increasing duration of dementia; however, this relationship was not statistically significantly in these 11 AD patients. Ranked correlations showed that increasing F_2-IsoP levels were significantly correlated with increasing Braak stage ($p < 0.001$), but not the number of ApoE4 alleles, for all 22 subjects. When analysis was restricted to AD patients or control subjects only, neither Braak stage nor the number of ApoE4 alleles was significantly correlated with F_2-IsoP levels.

Recently, we have described a series of F_2-IsoP-like compounds derived from peroxidation of docosahexaenoic acid[10]; because docosahexaenoic acid is found primarily in the central nervous system (CNS), we have termed these compounds F_4-neuroprostanes (F_4-NPs). There was sufficient VF available for analysis of F_4-NP levels in 4 of the AD patients and 3 control subjects. Indeed, average VF F_4-NP levels were 110 ± 12 pg/ml in these AD patients and 64 ± 8 pg/ml in control subjects ($p < 0.05$). VF F_2-IsoP and F_4-NP levels showed near perfect linear correlation in these 7 subjects ($r^2 = 0.97$, $p < 0.001$).

To establish the feasibility of determining CSF F_2-

Smokers

Name:	IPF2a - II	IPF2a - VI
Smoking	Smoker: non smoker:	Smokers: non smokers:
Hypercholesterolemia	HH: control:	

Table. Clinical, Pathological, and F_2-IsoP Data for Subjects with Postmortem Examination

	Age (yr)	Female/Male	Duration of Disease (yr)	Brain Weight (g)	Braak Stage	% of Alleles as ApoE4	F_2-IsoP (pg/ml)
Control (n = 11)	82.2 ± 1.8	8/3	0.0	1,233 ± 32	1.7 ± 0.4	12%	46 ± 4
AD (n = 11)	78.4 ± 1.6	7/4	7.2 ± 1.2	1,090 ± 51[a]	5.8 ± 0.1[c]	50%[d]	72 ± 7[b]

Data are mean ± SEM values, percentages of ApoE4 with respect to total numbers of ApoE alleles, or the numbers of male and female patients. Ages of AD patients and control subjects were not significantly different.

Unpaired *t* test yielded [a]$p = 0.05$, [b]$p = 0.01$, or [c]$p < 0.001$, for control subjects vs AD patients as indicated.

[d]χ^2 test with $p < 0.05$, for contingency table of presence of ApoE4 vs presence of AD.

F_2-IsoP = F_2-isoprostane; ApoE = apolipoprotein E; AD = Alzheimer's disease.

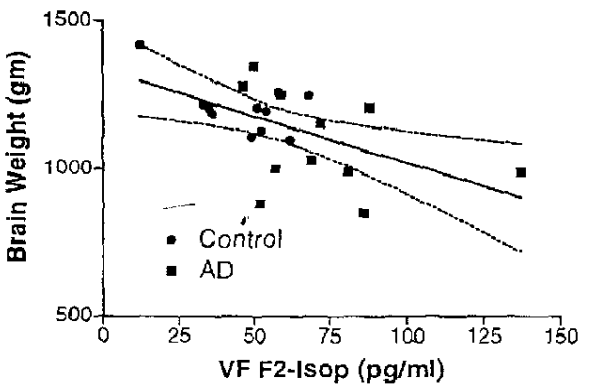

Fig. Scatter plot of VF F_2-IsoP concentration (pg/ml) versus brain weight (gm) for 22 control subjects and Alzheimer's disease patients with best-fit regression line and 95% confidence intervals ($r^2 = 0.32$, $p < 0.01$). VF = cerebrospinal fluid from lateral ventricles; F_2-IsoP = F_2-isoprostane.

IsoP levels during life, we also analyzed CSF aspirates from the lumbar cistern (LF) in 2 additional patients with suspected neurological disease but normal CSF. LF free F_2-IsoP levels in these 2 patients were 30 and 32 pg/ml, approximating the VF levels in control subjects and demonstrating the potential of measuring F_2-IsoP levels during life.

Discussion

AD is associated with increased lipid peroxidation in diseased regions of brain that have been studied post mortem. Although this approach has the advantage of coupling biochemical data with pathological verification of AD, two critical disadvantages have been that the assays used cannot be easily performed intra vitam and many are not entirely specific for lipid peroxidation. In the present study, we measured free F_2-IsoP concentrations, specific products of free radical–catalyzed peroxidation of arachidonic acid, in CSF from clinically and pathologically defined subjects. Our results showed that average VF F_2-IsoP levels in AD patients were significantly greater than in carefully documented control subjects. Moreover, VF F_2-IsoP levels were inversely correlated with brain weight. Also, in a limited manner, we demonstrated the feasibility of measuring F_2-IsoPs intra vitam in CSF aspirates from lumbar cistern. There was no correlation between VF F_2-IsoP levels and the number of ApoE4 alleles in our study; however, the number of patients was small and this lack of association with ApoE genotype must be addressed definitively in a larger series of patients.

In the present study, F_2-IsoP levels in VF from control subjects were similar to average plasma levels in healthy human volunteers,[5] suggesting that free F_2-IsoP may equilibrate between plasma and intrathecal compartments and that VF F_2-IsoPs in control subjects may be derived, at least in part, from plasma. However, several points support the contention that elevated VF F_2-IsoP levels in AD patients are derived from brain. First, numerous studies have consistently associated AD with regionally increased oxidative damage to brain[1] but have not consistently observed evidence of increased systemic oxidative stress.[1,13] Also, in the present study we demonstrated coincident elevations in VF F_4-NP and F_2-IsoP concentrations, the former being derived from docosahexaenoic acid that is extensively enriched in the CNS.[14]

We propose that CSF F_2-IsoP concentration may serve as a biomarker of CNS lipid peroxidation in patients with AD. We are not aware of any other quantifiable biomarker of AD that is significantly correlated with reduced brain weight, a manifestation of cerebral atrophy, and that may be measured during life. Quantification of CSF F_2-IsoP concentration may have use as an intra vitam index of disease progression or response to therapeutic intervention.

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Search for Varicella Zoster Virus in Giant Cell Arteritis

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Polymerase chain reaction and immunohistochemical analyses of formalin-fixed temporal arteries from 10 pathologically verified cases of giant cell arteritis did not reveal varicella zoster virus antigen or DNA.

Nordborg C, Nordborg E, Petursdottir V, LaGuardia J, Mahalingam R, Wellish M, Gilden DH. Search for varicella zoster virus in giant cell arteritis. Ann Neurol 1998;44:413–414

The cause of giant cell arteritis is unknown. Its acute to subacute nature, characteristic inflammatory pathology, including multinucleated giant cells, all suggest an infectious, particularly viral cause. Because varicella zoster virus (VZV) reactivates mostly in elderly humans (the same age group in which giant cell arteritis predominates), has a predilection for arteries,[1,2] and produces multinucleated giant cells in acutely infected tissue, many clinicians, especially neurologists, have questioned whether VZV causes giant cell arteritis. Thus, we analyzed arteries from 10 pathologically verified cases of giant cell arteritis for VZV antigen and DNA. Herpes simplex virus (HSV), a prototype herpesvirus that does not produce arteritis, was used as a control for our studies.

Materials and Methods

Temporal artery biopsies were obtained from 10 women with a clinical diagnosis of giant cell arteritis. Their mean age was 76.6 years (SD, 4.7 years). Arteries were fixed in 4% formaldehyde, cut in 1- to 3-mm-thick slices, dehydrated in graded alcohols, and embedded in paraffin. Five-micrometer-thick cross sections were stained with a combined van Gieson–elastin stain and examined by light microscopy. Arterial lesions were characterized by a chronic, mononuclear inflammatory reaction in the adventitia, media, and intima.

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